ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Study Of The Preservative Effects Of Carway *(Carm carvi L.)* Extracts And Use It On Prolong The Shelf Life Of Ground Meat.

Taghreed A AL-Nashi*, Ibtehaj M AL-Hakeem, and Nagham S AL-Awadi.

Faculty of Agricultural sciences, University of Sulaimani-Iraq.
Food sciences Department, University of Baghdad, Baghdad –Iraq.
Food sciences Department, Sulaymaniyah – Iraq.

ABSTRACT

In this study the antimicrobial activity of **Carway (*Carm carvi*)** extracts was evaluated against three species of Gram negative bacteria (*Pseudomonas putidi* , *Escherichia coli* ,and *Klebsiella pneumonia*)and two species of Gram positive bacteria (*Bacillus subitilus* and *Staphylococcus aureus*)and three species of molds (*Aspergillus flavus* , *Fusarium oxysporium* , and *Penicillium digitatum*). MIC, MBC and MFC of the extracts were determined at 37°. The aqueous and alcoholic extracts didn't have antibacterial and antifungal activity , while the essential oil has antimicrobial effects . Among the tested Gram-positive bacteria *Bacillus subitilus* was the most sensitive strain among the tested Gram-negative bacteria *Klebsiella pneumonia* was the most sensitive strain and among all bacteria tested in this study *Staphylococcus aureus* was the most resistible. The concentrations (2000 , 1000, 500 and 250 ppm) of essential oil has antifungal activity while the concentration 125 ppm showed simple inhibition activity on molds used and 60 ppm concentration has no antifungal activity on any of the fungi used in the study .The results obtained expanded the possibilities for application of studied oils not only as flavour enhancers, but even as natural antimicrobials in chilled meat products.

Keywords: *caraway seeds ,* ground meat*, Essentail oils , Carm carvi L. ,* natural antimicrobial*,* plant extract

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Constantly increasing requirements of people to consume natural and safety foods without additional chemical flavour enhancers and synthetic preservatives, influenced scientists and industrial producers to search for natural compounds with wide spectrum of antimicrobial activities. Among the natural substances essential oils from spices are the most appropriate and promising natural antimicrobials, because they manifest their antimicrobial activity even at low concentrations, do not cause microbial resistance and undesired changes in foods, also are GRAS and commonly accepted by consumers (4,8). In addition to antimicrobial properties, essential oils demonstrated antioxidant, antiviral, antimycotic, antitoxigenic, antiparasitic and insecticidal action (1,16). Psychrotrophic microorganisms associated with spoilage of chilled foods, especially meat and meat products, are one of the major problems of food industry. Microbial growth of spoilage and pathogenic psychrotrophic microorganisms and their metabolites caused undesired changes in organoleptic properties of foods and may be dangerous for human health (8).Antimicrobial activities of essential oils from spices such as *Origanum vulgare, Thym vilgaris, Satureja montana, Pimenta dioica* and other are widely studied, but usually they are tested *in vitro* at 30-35 °C, mainly against reference strains and by applying different antimicrobial testing procedures (4-6,8-14,18-22). On the other hand less or nothing is published about the antimicrobial action of essential oils from spices against food spoilage microorganisms at 4 °C. The mentioned reasons make many of the published results to a certain extent incomparable and impracticable in real foods and real industrial conditions. The objectives of the present study were to evaluate the antimicrobial activity of Caraway *(Carm carvi)* seeds essential oils and aqueous extracts against some species of bacteria and mold to use it as preservative to prolong the shelf life of ground meat.

MATERIALS AND METHODS

Collection of plant materials:

Carway *(Carm carvi)* seeds were randomly collected and stored in deep freeze at −20 ◦C until analysis.

Chemicals, solvents, reagents

All solvents ,chemicals and reagents were purchased from Aldrich Co. and Sigma Chemical Co. and were of analytical grade.

Preparation and extraction of the plant materials Plant materials were ground and macerated for extraction. Twenty grams of each prepared sample were weighed into 1 L Erlen-meyer flasks, and then 200 ml of solvents (ethanol and water) were added to the samples. Extraction was carried out by shaking at room temperature for 72 h. After filtration through filter paper (Whatman No. 4), the residue was re-extracted twice, and then the combined extracts of every sample were evaporated at room temperature and dried to a constant weight. The final residues were used to study their antimicrobial activities (18).

Isolation of essential oil Portions (100 g) of each prepared plant material were hydro-distilled for 6 h in a Clevenger type apparatus to isolate the essential oil. The obtained essential oils were dried over anhydrous sodium sulphate. After filtration they were stored in dark glass bottles at −4 ◦C until used for further analyses.

Microorganisms

Three species of Gram negative bacteria (*Pseudomonas putidi , Escherichia coli ,and Klebsiella pneumonia*) and two species of Gram positive bacteria (B. *subitilus* and *Staphylococcus aureus* and three species of mold (*Aspergillus flavus , Fusarium oxysporium , and Penicillium digitatum*) were used as indicator microorganisms for detection of the antimicrobial activity. All strains mentioned above were obtained from the Food sciences and biotechnology Dep., College of Agriculture, Baghdad University.

Chemical analysis of caraway seeds:

Moisture content , ash , and lipid were determined according to AOAC (2). Protein content was determined using the Kjedhal method AOAC (2).

Bioactive compound analyses in caraway extract

Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses.

Alkaloids determined using Wagner test (5) , Cardiac glycosides according to Kellar Kiliani test(17), flavoniods using Shinoda test (12) , phenols using Phenol test (9) , saponine according to Frothing test / Foam test,(17) , tannins usings Braemer's test(12); (17)

Determination of antibacterial activity

Disc diffusion method

The disc diffusion method described by Parekh,J. *et al*. (17) was used for determination of antimicrobial activity of plant extracts and their essential oils, as follows: sterile nutrient agar medium (Merck) was prepared and distributed into Petri plates of 90 mm diameter. The disc diameter used was 6 mm (Whitman No. 1) paper. Different dilutions of the extracts and essential oils were made with ethanol. The microbial suspension was spread over the surface of the nutrient agar using a L-shaped glass rod. Under aseptic conditions, the discs were placed on the agar plates and then 200, 500, 1000, and 2000 ppm from each of the extracts and essential oil dilutions was put on the discs. A dilution solvent (ethanol) was added to the discs on the control plates. The plates were then incubated at optimum temperature 30 ◦C for bacteria) for 24–48 h in order to get reliable microbial growth. Diameters of microbial inhibition zones (mm) were measured and recorded.

Determination of antifungal activity

Five millimeter disk of each tested fungi was transferred to the center of PDA dish supplemented with different concentrations (200 , 500 , 1000, 2000 ppm)of plant extracts and essential oil . The control treatment was inoculated in PDA dish without extract at the same conditions. Both experimental and control dishes were incubated at 25C and the fungal growth diameter was measured after the fungal growth in the control treatment had reached the edge of the petri dish (7). This test was repeated three times and the inhibition was calculated as the percentage reduction in colony diameter growth compared with the control for each species.

Minimum inhibitory concentration (MIC) of plant extracts and essential oils

A micro dilution broth susceptibility assay was used as recommended by Natural Committee for Clinical Laboratory Standard-NCCLS for the determination of the MIC. All tests were performed in nutrient broth for bacteria and in potato dextrose broth for fungi. Concentrations of 200, 500, 1000 and 2000 ppm of dry extract or essential oil were added to 1 ml nutrient broth tubes containing 10^5 CFU/ml of live microorganism's cells. The tubes which contained 10 ml broth were incubated on an incubator shaker as to evenly disperse the extracts and essential oils throughout the broth in the tubes. The highest dilution (lowest concentration), showing no visible growth, was regarded as MIC. Cells from the tubes showing no growth were subcultured on nutrient agar plates to determine if the inhibition was reversible or permanent.

Essential oil in ground meat preservation

Fresh ground meat was purchased from the local grocery shop and the initial numbers of the total aerobic bacterial count, psychrotrophic bacteria, coliform and yeasts and molds were estimated. The ground meat was divided into four equal groups and one group for control. The essential oil was added and to obtain

the final concentrations (200.500, 1000 , and 2000) mg / kg (weight / weight) the essential oil distributed by using sterile glass rod until homogeneity of essential oil with ground meat . The ground meat was covered to prevent volatilization of essential oil and kept in the refrigerator at 4C.

The total aerobic bacterial count , psychrotrophic bacteria , coliform , and yeasts and molds were estimated at a different time (1 , 4, 8, 12 , 22 days) .

RESULTS AND DISCUSSION

Bioactive compound analyses in caraway extract as shown in Table 1 the fruits of caraway plant containd flavonoids, phenols, saponine and tannins. Flavoniods possess antifungal and antiviral properties

Table 1: Chemical composition of Carway *(Carm carvi)* seeds

Protein	24.00 %
Ash	3.50 %
Oils	9.60 %
Moisture	7.00 %

Table 2: Bioactive compound in caraway extract

Bioactive compound	result
Alkaloids	–
Cardiac glycosides	–
flavoniods	+
phenols	+
saponine	+
tannins	+

Essential oil content

The yield of hydrodistilled carway seeds essential oil was found to be 3.50%. The factors involved in the essential oil content and composition are: the characteristics and the tillage of soil, the annual precipitation and fertilization amount, breeding, the maturation, harvesting, drying and the extraction technologies (11), and even the microelement content (heavy metals) of the soil (1),(21) found that caraway fruits contain 1–9% of essential oils dependent on planting conditions .

Antimicrobial activity of essential oils and plant extracts

Estimation of antimicrobial activity of essential oils and plant extracts by disc diffusion method. Food poisoning originating from contaminated foods by both Gram-positive and Gram-negative bacteria causes concern to society and to industry. A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency of antimicrobial treatment (20). Spices and herbs have been safely since ancient times as food flavoring agents and also as herbal medicines and are now mainly considered “generally regarded as safe” (GRAS). Recently there have been considerable emphasis studies involving essential oils and extracts of spices and herbs on inhibiting the growth of microbes .Gianni *et al*. (9) reported that the essential oil and seed extracts of caraway could be a source of natural antimicrobial materials required for the preparations of new food preservatives. Therefore, this piece of work has been undertaken and used the disc diffusion technique to estimate the antimicrobial activity of essential oils and different extracts of caraway. The antimicrobial activity of the essential oils and plant extracts were examined in the disc diffusion assay against three species of Gram negative bacteria (*Pseudomonas putidi , Escherichia coli* ,and *Klebsiella pneumonia*)and two species of Gram positive bacteria (*Bacillus subitilus* and *Staphylococcus aureus*)and three species of molds (*Aspergillus flavus* , *Fusarium oxysporium* , and *Penicillium digitatum*). The data expressed as diameter of growth inhibition zone (mm). The results are illustrated in Tables 3.

ISSN: 0975-8585

Table 3: Antimicrobial activity of plants extracts using disc diffusion assay (inhibition zone diameter in mm).

Conc. ppm/disk	Microorganisms				
	E. coli	S. aureus	B. subitilus	P.putidia	K.pneumoniae
200	5.6	4.6	5.2	5	5
500	15	4.7	15	14	15
1000	21	12	17	16	21
2000	22	18	19	19	23

The diameter (mm) of the inhibition zone is the mean of three independent experiments including the diameter of the paper disc (4 mm) .

Variable zones of microbial growth inhibition were noted in essential oils. Zones of growth depend on the concentration of essential oil. The concentration activity, Caraway essential oil exhibited considerable antimicrobial activity against all the strains tested, particularly against Gram-negative bacteria. Eethanol extract and water extract were the 2000 ppm/disc was the best concentration as antimicrobial lower effect as antimicrobial activity than essential oil.

Determination of antimicrobial activity of essential oils by A micro dilution broth susceptibility assay method . (MIC) The minimal inhibitory concentration (MIC) of caraway essential oil and their extracts were determined in order to assess their antimicrobial activity. The MIC values of caraway essential oil , (1000 ppm), for *Pseudomonas putidi , Escherichia coli ,and Klebsiella pneumonia* , and B. *subitilus* while *S. typhi* required higher concentration (2000 ppm) .In this study, the essential oil displays effective antimicrobial effects against food spoilage and food-borne pathogens and broad antimicrobial spectrum.

Table 4: Antifungal activity of caraway essential oil

Conc. ppm	molds growth diameter mm		
	Aspergillus flavus	*Fusarium oxysporium*	*Penicillium digitatum*
200	no antifungal activity	no antifungal activity	no antifungal activity
500	70	69	75
1000	30	23	44
2000	10	8	13

As shown in 4 the caraway essential oil exhibited high antifungal activity against all strains tested at concentration 250 mg/kg .The mode of active of essential oils is a result of attack of oil on the cell wall and reaction of cytoplasm in the hyphae and ultimately death of the mycelium (22).

Table 5: The effects of Caraway essential oil (200, 500, 1000, and 2000 mg/ kg) on psychrotrophic bacteria on ground meat at 4°C by time (days)

Caraway essential oil concentrations (mg/kg)	Time (days)				
	Microbial growth (cfu/g) on ground meat treated with caraway essential oil				
	1	4	8	12	22
200	55 x10	60 x10	62 x10	22 x 10^2	33 x 10^2
500	34 x10	41 x**10**	42 x**10**	13 x 10	83 x 10
1000	52 x**10**	50 x**10**	50 x**10**	52 x**10**	36 x 10
2000	50	50	60	61	22 x 10
Cont rol	14 x 10^2	32 x 10^2	90 x 10^2	22 x 10^3	98 x10^6

ISSN: 0975-8585

The original microbial load (mesophilic) of minced meat was 5 x 10^3 cfu/g used as a control. The gram-negative psychrotrophic bacteria (PTC) are the major group of microorganisms that is responsible for aerobic spoilage of stored fish at chilled temperatures (4 ± 1C) (21) . At the end of storage time (22th day), PTC in control samples was the highest (98 x 10^6cfu/g) and a lower count was detected in samples treated with 2000 mg/kg the reason may be due to the strong antimicrobial activity of Caraway essential oil .

Table 6: The effects of Caraway essential oil (250, 500, 1000, and 2000 mg/ kg) on coliform bacteria on ground meat at 4°C by time (days) .

Caraway essential oil concentrations (mg/kg)	Time (days)				
	Microbial growth (cfu/g) on ground meat treated with caraway essential oil				
	1	4	8	12	22
200	0 colonies	11x10	20x10	36 x 10	90 x 10
500	12 colonies	16 colonies	16 colonies	15colonies	13colonies
1000	0 colonies	0 colonies	0 colonies	0 colonies	0 colonies
2000	0 colonies	0 colonies	0 colonies	0 colonies	0 colonies
Control	10 x 10	16 x 10^2	30 x 10^2	22 x 10^3	77 x 10^3

As shown in table 6 coliform bacteria counts in control group at the end of storage time (22 days) was the highest (77 x 10^3)CFU.g-1. Followed by group treated with 250 mg/kg of caraway essential oil (90 x 10) CFU.g-1. Also, the growth of coliform bacteria were inhibited in groups treated with 500, 1000, and 2000 mg/kg caraway essential oil.

Generally, the essential oils present in plant matter have been attributed as principal sources of compounds exhibiting antimicrobial activity, which has been illustrated against bacteria and fungi (6)

Table 7: The effects of Caraway essential oil (200, 500, 1000, and 2000 mg/ kg) on molds and yeasts on ground meat at 4°C by time (days).

Caraway essential oil concentrations (mg/kg)	Time (days)				
	Microbial growth (cfu/g) on ground meat treated with caraway essential oil				
	1	4	8	12	22
250	67	77 x**10**	89 x10	91 x10	95 x10
500	70	80	82	90	93
1000	44	49	55	56	60
2000	23	32	34	45	45
Control	12 x**10**	22 x10^2	80 x10^2	22 x 10^3	80 x 10^4

The antimicrobial activity of Caraway essential oil exhibited a stronger growth inhibiting effect against fungi than bacteria Table 6. At lower concentrations (250 mg/kg) of Caraway essential oil the growth of molds and yeasts were inhibited.

The fungicidal activity of the oil was observed at a concentration, that is Inouye and others (10) reported that hydrophobic compounds of essential oils are thought to be absorbed by highly lipophilic nature

of fungal mycelia. Also, it has been reported that essential oils revealed alterations in the morphology of the hyphae and in the sporulation process (11). The mode of active of essential oils is a result of attack of oil on the cell wall and reaction of cytoplasm in the hyphae and ultimately death of the mycelium (22).

CONCLUSIONS

The essential oils from caraway demonstrated antimicrobial activity against all of the tested microorganisms used in this study which expand the possibilities for application of this oil not only as flavour enhancers, but even as natural antimicrobials in chilled ground meat.

ACKNOWLEDGMENT

We thank the department of Food Science and Biotechnology, College of Agriculture /University of Baghdad for their generous gift of the bacteria (*Pseudomonas putidi, Escherichia coli and Klebsiella pneumonia* B. *subitilus* and *Staphylococcus aureus* and molds (*Aspergillus flavus*, *Fusarium oxysporium and Penicillium digitatum*)

REFERENCES

[1] Abu-Darwish M.S., Ofir R., (2014), Heavy metals content and essential oil yield of Juniperus phoenicea L. in different origins in Jordan, Environmental Engineering and Management Journal, 13, 3009- 3014.
[2] AOAC International. (2005).Official methods of analysis of the Associated of official analytical chemists.18th edition ed. AOAC International, Arlington
[3] Bedin, C., Gutkoski, S.B. and Wiest, J. M. (1999) Higiene Alimentar, 13, 26-29
[4] Burt S. (2004) Essential oils; their antimicrobial properties and potential applications in foods-a review. International journal of food microbiology. 94; 223-253.
[5] Chanda S V, Parekh J, Karathia N. Evaluation of antibacterial activity and phytochemical analysis of Bauhinia variegate. L bark Afr J Biomed Res. 2006;9:53–56.
[6] Davidson, P. M., Critzer, F. J., Taylor, T. M. 2013. Naturally occurring antimicrobials for minimally processed foods. *Annual Review of Food Science and Technology*, vol. 4, p. 163-190. http://dx.doi.org/10.1146/annurev-food-030212-182535.
[7] Ferdes¸ M, Ungureanu C. 2012. Antimicrobial activity of essential oils against four food-borne fungal strains. UPB Sci Bull Series B 77:87–98.
[8] Girova, T., Gochev, V., Jirovetz, L., Buchbauer, G., Schmidt , E.,and Stoyanova, A.(2014). Antimicrobial activity of essential oils from spices against psychrotrophic food spoilage microorganisms. in Biotechnology & Biotechnological Equipment 24(2):547-552 ·
[9] Gianni S., Silvia M. a, Mariavittoria M., Martina S.,Stefano M., Matteo R., Renato B. (2005) Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry ,91 ,621–632.
[10] Inouye S, Tsuruoka T, Watanabe M, Takeo K, Akao M, Nishiyama Y, Yamaguchi H. 2000. Inhibitory effect of essential oils on apical growth of *Aspergillus fumigatus* by vapour contact. Mycoses 43:17–23.
[11] Kolta R., Hornok L., (1992), Extraction processes for essential oils, In: Cultivation and Processing of Medicinal Plants, Hornok L. (Ed.), John Wiley & Sons, Chichester, 93-104.
[12] Kumar G S, Jayaveera K N, Kumar C K A, Sanjay U P, Swamy B M V, Kumar D V K.(2007) Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Trop J. Pharm. Res.;6:717–723.
[13] Ouattara B. , Simard R.D. , Holley R.A., Piette G.J.P. and Bégin A. (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int J Food Microbiol., 37, 155-162
[14] Lis-Balchin, M., S. Hart, S. G. Deans, and E. Eaglesham. (1996) J Herbs, Spices and Medicinal Plants, 4, 69-86.
[15] Najda A, Dydch J, Brzozowski N.2008. Flavonoid content and antioxidant activity of caraway roots (*Carum carvi* L.). Vegetable crops research bulletin . vol. 68, 127-133.
[16] Ouattara B. , Simard R.D. , Holley R.A., Piette G.J.P. and Bégin A. (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int J Food Microbiol., 37, 155-162
[17] Parekh J, Chanda S V.(2007) In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol.;31:53–58.

[18] Pizzale, L., Bortolomeazzi, R., Vichi, S., Conte, L.S., 2002. Antioxidant activity of sage and oregano extracts related to their phenolic compound content. J. Sci. Food Agric. 82, 1645–1651.

[19] Sacchetti,G., Maietti,S., Muzzoli,M., Scaglianti,M., Manfredini, S., Radice, M.,and Bruni,R.(2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry 91 ,621–632.

[20] Sallam,K.I., Ahmed, A.M., ELGazzar, M.M. and ELDaly, E.A. 2007. Chemical quality and sensory attributes of marinated Pacific saury (*Cololabis saira*) during vacuum-packaged storage at 4°C. Food Chem. *102*(4),1061–1070.

[21] Sedláková j., Kocourková S., Lojková L., Kubáň V. (2003). Determination of essential oil content in caraway (Carum carvi L.)species by means of supercritical fluid extraction. Plant Soil Environ., 49, (6): 277–282.

[22] Sharma N, Tripathi A. 2007. Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) van Tieghem. Microbiol Res 163:337–44.